STUDIES IN BLOOD PRESERVATION

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I. REPARTITION OF POTASSIUM IN CELLS AND PLASMA

Dulière [1] reported in 1931 a constant enrichment in the serum potassium of blood kept in contact with cells over a period of days.

The manifestations of potassium poisoning have been well known since Blake's [2] observation in 1840. The symptoms in both cold [3] and warm [4] blooded animals have been reported. Such poisoning is not peculiar to the animal kingdom, for alterations in the concentration of potash in certain plant cells or in the mediums in which they live profoundly affect many of their normal reactions.[5]

Because of this, an investigation of the loss of potassium from cells and its increase in the plasma, as one of the abnormalities of stored blood, was felt necessary. An investigation seemed doubly imperative on account of the recent rapid rise of "blood banks," together with the growing conviction that blood kept too long is dangerous, as judged by an occasional severe reaction, a greater incidence of post-transfusion jaundice and an occasional unexplained death.

Normally human red cells contain twenty times as much potassium as there is in the plasma. It is the chief mineral base of cells. Naturally factors altering this distribution would make available an ever increasing amount of potassium in the serum of preserved blood.

FIRST SERIES

The first group of experiments was designed to check Dulière's statement. Two samples of the same human venous blood were collected with aseptic precautions and kept in pyrex flasks stoppered with cotton, in the dark, in a refrigerator at 4 C. in the following manner:

EXPERIMENT 1.—The blood, 250 cc., was kept under liquid petrolatum.

EXPERIMENT 2.—The blood, 250 cc., was mixed with 2.5 per cent solution of sodium citrate in sufficient quantity to make a mixture containing 0.31 Gm. per hundred cubic centimeters of blood.

At the same time a 5 cc. sample was mixed with heparin (Connaught) in a centrifuge tube and spun for one hour. This served as the sample for base line determinations.

Portions of the serum or plasma were pipetted off at twenty-four hour intervals for five days and then at approximately weekly intervals for a month. With each analysis, material for culture was taken and streaked on blood agar plates. These were observed for growth at the end of twenty-four and forty-eight hours.

Potassium determinations were done by the argenticobaltinitrite modification [6] of the method of Kramer and Tisdall,[7] the final colorimetric readings being made on the Evelyn photoelectric colorimeter.[8] The value given is the mean of two aliquots. Cell volume was determined by Sanford-Magath tubes spun for one hour at 2,000 revolutions per minute.[9] Specific gravity was measured by the method of Barbour and Hamilton.[10] The plasma protein content was calculated by the formula of Weech, Reeves and Goettsch.[11]

The following experiment is typical of the series as to method and results:

Dr. Drew is a Fellow in Surgery, Rockefeller Foundation.
This study was made possible by a grant from the Blood Transfusion Betterment Association.
From the Department of Surgical Pathology of Columbia University College of Physicians and Surgeons
Data presented at the Eleventh Annual Graduate Fortnight, the New York Academy of Medicine, Oct. 24-Nov. 4, 1938.
Owing to lack of space, this article has been abbreviated for publication in THE JOURNAL by the omission of several tables. The complete article appears in the authors' reprints.

1. Dulière, W. L.: Données expérimentales sur la répartition du potassium dans le sang extravasé; comparaison entre la concentration physiologique dans le plasma et la concentration dans le liquide céphalorachidien, Compt. rend. Soc. de biol. **108**: 416-418 (Oct. 23) 1931.

2. Blake, James: Observations and Experiments on the Mode in Which Various Poisonous Agents Act on the Animal Body, Edinburgh M. & S. J. **53**: 35-49, 1840; On the Action of Saline Substances When Introduced into the Vascular System, ibid. **54**: 339-349, 1840.

3. Botazzi, P.: Sur le mécanisme d'action des sels de potassium sur le cœur, Arch. de physiol. norm. et path. **8**: 882-892, 1896. Bouchardat and Stuart-Cooper.[12] Binet.[37]

4. Beck, A.: Ueber die Giftwirkung des Harnes, Arch. f. d. ges. Physiol. **71**: 560-595, 1898. Braun, L.: Ueber die Wirkung der Kalisalze auf das Herz und die Gefässe von Saugethieren, ibid. **103**: 476-492, 1904. Bunge, Gustav: Ueber die Bedeutung des Kochsalzes und das Verhalten der Kalisalze im menschlichen Organismus, Ztschr. f. Biol. **9**: 104-143, 1873. Falck, F. A.: Experimentelle Studien zur Beschaftung der Temperaturcurven der acuten Intoxicationen, Virchows Arch. f. path. Anat. **51**: 519-552, 1870. Gottlieb, Rudolf: Ueber die Wirkung des Nebennierenextractes auf Herz und Gefässe, Arch. f. exper. Path. u. Pharmakol. **43**: 287-304, 1900. Guttmann, Paul: Expermientelle Untersuchungen über die Wirkung der Kali- und Natronsalze, Berl. klin. Wchnschr. **2**: 344-348, 355-358, 367-371, 1865; Ueber die physiologische Wirkung der Kali- und Natronsalze mit Rücksicht auf die Untersuchungen des Herrn Dr. Podcopaew in Petersburg, Virchows Arch. f. path. Anat. **35**: 450-469, 1866. Herringham, W. P.: An Account of Some Experiments upon the Toxicity of Normal Urine, J. Path. & Bact. **6**: 158-179, 1900. Olmer, D.; Payan, L., and Berthier, J.: Teneur en potassium des divers organes, chez le chien normal et dans l'intoxication par le chlorure de potassium, Compt. rend. Soc. de biol. **89**: 330-332, 1923. Podkopaeff, Fedor: Vergleichende Untersuchungen über die Wirkung des Chlorkalium und Chlornatrium auf den thierischen Organismus, Virchows Arch. f. path. Anat. **33**: 505-517, 1865. Richet, Charles Robert: De l'action physiologique des sels de rubidium, Compt. rend. Acad. d. sc. **101**: 667-669, 1885. Bernard.[39] Traube.[39] Grandeau.[21] Aubert and Dehn.[22] Bohm.[23] Dogiel.[24] Feltz and Ritter.[25] Bochefontaine.[26] Mathison.[29] Kleeberg.[41] Bunge.[42] Bouchard and Oliver.[28] Joseph and Meltzer.[24]

5. Osterhout, W. J. V.: Some Fundamental Problems of Cellular Physiology, New Haven, Yale University Press, 1927; The Absorption of Electrolytes in Large Plant Cells, Bot. Rev. **2**: 283-315, 1936.

6. (*a*) Breh, F., and Gaebler, O. H.: The Determination of Potassium in Blood Serum, J. Biol. Chem. **87**: 81-89 (May) 1930. (*b*) Truszkowski, Richard, and Zwemer, R. L.: Cortico-Adrenal Insufficiency and Potassium Metabolism, Biochem. J. **30**: 1345-1353 (Aug.) 1936. (*c*) Determination of Blood Potassium, ibid. **31**: 229-233 (Feb.) 1937.

7. Kramer, B., and Tisdall, F. F.: A Clinical Method for the Quantitative Determination of Potassium in Small Amounts of Serum, J. Biol. Chem. **46**: 339-349 (April) 1921.

8. Evelyn, K. A.: A Stabilized Photoelectric Colorimeter with Light Filters, J. Biol. Chem. **115**: 63-75 (Aug.) 1936.

9. Sanford, A. H., and Magath, T. B.: A New Centrifuge Tube for Volume Index Determinations (Modified Haden Method), J. Lab. & Clin. Med. **15**: 172-173 (Nov.) 1929.

10. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity, J. Biol. Chem. **69**: 625-640 (Aug.) 1926.

11. Weech, A. A.; Reeves, F. B., and Goettsch, E.: The Relationship Between Specific Gravity and Protein Content in Plasma, Serum and Transudate from Dogs, J. Biol. Chem. **118**: 167-174 (Feb.) 1936.

Experiment 1.—*Blood kept under oil without a preservative.* The blood of the donor, Dr. J. S., was of group B.

Procedure.—In addition to the determinations, which served to provide basic values, made at the time of the bleeding, eight other sets of analyses were made, and the results were expressed in four ways (table 1):

1. Column 4; actually observed; serum potassium as milligrams per hundred cubic centimeters of serum.

2. Column 5; by calculation as milligrams of potassium in the serum of 100 cc. of blood. This figure for practical purposes gives at a glance the actual amount of serum potassium in every hundred cubic centimeters of blood on any given day when preserved in the stated manner.

3. Column 6; by calculation as milligrams of potassium given off into the serum by each hundred cubic centimeters of cells. This figure is theoretically more convenient for comparative purposes, as it obviates the differences created by bloods whose cell volumes may vary markedly from normal.

4. Column 7; as percentage of potassium which has diffused out of the cells.

Table 1 (experiment 1).—*Blood Kept Under Oil Without Preservative*

1	2	3	4	5	6	7	8
			Milligrams of Potassium			Percentage of Cell Potassium Diffused Out	
				Sum of Increments			
Date	Sample	Days	Observed Value per 100 Cc. of Serum*	Per 100 Cc. of Blood	From 100 Cc. of Cells		Hemolysis (Observed)
3/27/38	1	1	40.0	8.7	16.0	4.5	0
3/28/38	2	2	68.6	21.1	39.2	11.0	0
3/29/38	3	3	83.0	27.2	51.0	14.2	0
3/30/38	4	4	100.8	35.1	64.7	18.3	0
3/31/38	5	5	133.2	48.6	89.7	25.3	0
4/ 9/38	6	14	200.0	76.1	140.5	39.6	++++
4/15/38	7	20	206.4	78.6	145.2	40.9	++++
4/29/38	8	34	225.0	86.0	158.0	44.8	++++

* Values uncorrected for sample removed.

5. Column 8; as hemolysis observed.

Each day's values represent the sum of the increments to that particular day, care being taken throughout to estimate the amount of potassium removed in the various test samples. At each sampling a note was made concerning the presence and degree of hemolysis.

Values.—

Basic Values: hematocrit reading, 54.3 per cent cells and 45.7 per cent serum; plasma potassium, 21 mg., whole blood potassium, 202 mg. and cell potassium (calculated), 354 mg., per hundred cubic centimeters; specific gravity of plasma, 1.0274, and plasma proteins, 6.87 Gm. per hundred cubic centimeters.

Calculation:

(*a*) For base line or zero values: blood sample, 250 cc.; serum volume, 250×0.457 (percentage of serum) $= 114.4$ cc.; determined serum potassium, 21 mg. per hundred cubic centimeters, and total potassium in original serum, 24 mg.

(*b*) For first day's increment: blood sample, 2 cc.; serum potassium (observed value), 40.0 mg. per hundred cubic centimeters; potassium in 2 cc. sample, 0.8 mg.; potassium in residual serum, $114.4 - 2 \times 0.4 = 45$ mg.; total potassium in serum after twenty-four hours, $45 + 0.8 = 45.8$ mg.; increase in total potassium in serum in twenty-four hours, $45.8 - 24$ (originally present) $= 21.8$ mg.; increase in serum potassium of 100 cc. of blood in first twenty-four hours, $21.8 \times \frac{100}{250 \text{ (cc.)}} = 8.7$ mg.; amount of potassium given off in first twenty-four hours by each hundred cubic centimeters of cells, $21.8 \times \frac{100}{250 \text{ (cc. of blood)} \times 0.543 \text{ (percentage of cells)}} = 16$ mg., and percentage of cell potassium diffused out into serum in first twenty-four hours, $\frac{16 \text{ (amount lost)}}{354 \text{ (original cell potassium)}} = 4.5$.

In a similar manner each day's results were determined and expressed as the sum of the increments.

Results.—These are shown in chart 1 and table 1.

In each of the two flasks there was a steady rise in the serum or plasma potassium. In the hematocrit tube, however, for the few days observed, the increment was less and suggested that either heparin was a markedly superior preservative or the slow diffusion was the result of some other factor.

In each instance there was discernible discoloration of the supernatant fluid by the fifth day and obvious gross hemolysis by the fourteenth.

The data in table 1 suggest that blood kept under oil at a constant temperature loses in the first week at least 25 per cent of its cell potassium, at the end of three weeks about 40 per cent and from that time on diminishing quantities. In more practical terms, such blood at the end of the first week contains in the serum of each hundred cubic centimeters of blood at least 50 mg. of potassium and any time after two weeks at least 75 mg., the amount gradually increasing.

SECOND SERIES

The second group of experiments was designed (1) to check the results of the first series, (2) to ascertain the effect of trauma (such as shaking) on the rate of loss of potassium from cells and (3) to see what effect the shape of the container had on the rate of diffusion. The blood of the donor, Dr. G. S., was of group O. Five hundred cc. of blood was collected and placed in three pyrex flasks.

Experiment 3.—The blood, 150 cc., was kept under oil without an anticoagulant.

Experiment 4.—The blood was placed in a Sanford-Magath hematocrit tube containing heparin and spun for an hour.

Experiment 5.—The blood, 150 cc., was mixed with 50 mg. of heparin.

Experiments 6 and 6B.—The blood, 200 cc., was mixed with the usual sodium citrate solution.

These flasks were treated in a manner similar to the first set. The results are shown in table 2.

From the observed values, none of the anticoagulants prevented the loss of potassium from the cells. The rate of diffusion appeared a little slower in the citrated blood. There was a distinct difference in the heparinized bloods (chart 2), that in the container with the larger interface showing the more rapid diffusion. This indicates that the shape of the container and not the anticoagulant was probably the cause of the slow diffusion in the heparinized blood in the first series. Here again, agitation by vigorous shaking hastened the process of diffusion (chart 3).

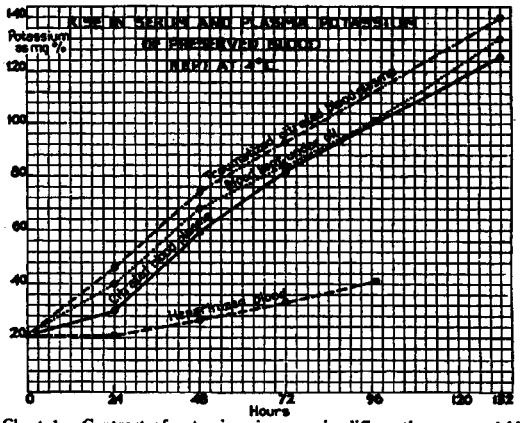

Chart 1.—Contrast of potassium increase in differently preserved bloods. Both the citrated blood and the blood stored under oil were in Erlenmeyer flasks. The heparinized blood was kept in a centrifuge tube. Data from experiment 1, table 1.

THIRD SERIES

In the third series four samples of fresh venous whole blood were preserved in 2.5 per cent solution of sodium citrate, 3 per cent solution of sodium citrate, Peyton Rous solution [12] and the Russian citrate solution.[13]

EXPERIMENT 7.—*Blood preserved in 2.5 per cent solution of sodium citrate.* The blood, from a professional donor, G. W., was of group O. To 125 cc. was added 17.5 cc. of 2.5 per cent solution of sodium citrate to give a mixture containing 0.31 Gm. per hundred cubic centimeters of blood. Each day a 2 cc.

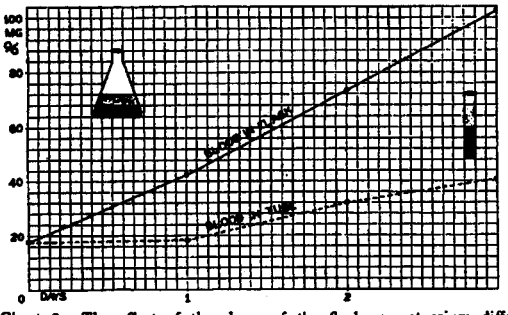

Chart 2.—The effect of the shape of the flask on potassium diffusion. Data from experiments 4 and 5, table 2.

sample was removed from the supernatant fluid without disturbing the cells and the potassium content determined as in experiment 1.

The results are tabulated in table 3 and graphically represented in charts 4 and 5.

EXPERIMENT 8.—*Blood preserved in 3 per cent solution of sodium citrate.* The donor was the same as in experiment 7 and the procedure was the same except that 15 cc. of a 3 per cent solution of sodium citrate in sterile water was used as the preservative for 125 cc. of blood. The gram percentages are exactly the same, 0.31.

The results are tabulated in table 4 and graphically represented in chart 5.

EXPERIMENT 9.—*Blood preserved in Russian citrate compound.* The blood of the donor, Dr. C. R. D., was of group O. Fifty cc. was added to an equal quantity of preservative made up according to the following formula: sodium chloride 7 Gm., sodium citrate 5 Gm., potassium chloride 0.2 Gm., magnesium sulfate 0.004 Gm. and distilled water 1,000 cc. The rate of diffusion was determined as in experiments 7 and 8.

The results are tabulated in table 5 and graphically represented in chart 5.

EXPERIMENT 10.—*Blood preserved in Peyton Rous compound.* The blood of the donor, Dr. J. S., was of group B. To 150 cc. was added 250 cc. of 5.4 per cent solution of dextrose in distilled water and 100 cc. of 3.8 per cent solution of sodium citrate. The high percentage of sodium citrate in this preservative contraindicates its use for transfusions; the cells, however, may be resuspended in saline solution and used. The results are shown in table 6 and chart 5.

SUMMARY, PART I

1. There is a daily increase in the amount of potassium present in the serum or plasma of whole blood kept in vitro under aseptic bacteriostatic conditions.

2. The transference of potassium from cells to plasma begins at the time of withdrawal from the blood stream. The rate is rapid at first and gradually diminishes.

3. The total amount found in the serum at the end of ten days reaches 25 per cent of the total potassium content of the red blood cells in the fresh state and at the end of thirty days may exceed 50 per cent.

4. The rate at which the potassium is given up by the cells is greatly increased by shaking.

5. The rate of potassium diffusion is evidently influenced by the shape of the container. The larger the interface area, the more rapid the diffusion (chart 2).

6. Hemolysis appeared at varying times in the different samples; none was present in the sample preserved in the Peyton Rous compound.

7. Changes observed in these experiments were not due to bacterial infection.

8. Sodium citrate in a mixture containing 0.31 Gm. per hundred cubic centimeters of blood is more effective as a preservative than the more complex Russian citrate compound.

9. The citrate-dextrose mixture of Peyton Rous prevents loss of hemoglobin from the cells but not loss of potassium.

II. A COMPARISON OF THE RATES AT WHICH CELLS LOSE POTASSIUM AND HEMOGLOBIN

In the third series of experiments, repeated attempts to measure accurately the small amounts of hemoglobin in the plasma of only slightly hemolyzed bloods were unsuccessful with the usual acid hematin methods such as those of Helige and Sahli. With the Pulfrich photometer, however, small daily increments were demonstrable with greater consistency. The method used is a modification of that described by Heilmeyer.[14] The content of hemoglobin may be calculated in grams per hundred cubic centimeters of plasma and then reexpressed as grams lost from the cells per hundred cubic centimeters of blood.

The potassium determinations were done as described in part I, the cultures on blood agar plates and the

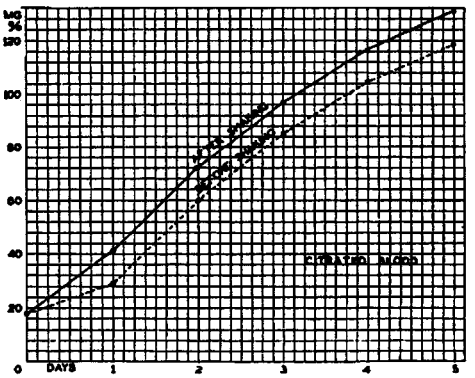

Chart 3.—Effect of shaking on preserved blood. Data from experiments 6 and 6B, table 2.

p_H determinations first by titration and then checked by means of a Helige glass electrode potentiometer which had been calibrated by buffers.

EXPERIMENT 11.—*Blood preserved in diluted Russian citrate compound.* The blood of the professional donor, J. F., was of group O.

Equal parts of blood and diluted preservative were mixed by adding 125 cc. of blood to 125 cc. of preservative which con-

12. Rous, Peyton, and Turner, J. R.: The Preservation of Living Red Blood Cells in Vitro: I. Methods of Preservation, J. Exper. Med. 23: 219-237 (Feb.) 1916.

13. Goodall, J. R.; Anderson, F. O.; Altimas, G. T., and MacPhail, F. L.: An Inexhaustible Source of Blood for Transfusion and Its Preservation, Surg., Gynec. & Obst. 66: 176-178 (Feb.) 1938.

14. Heilmeyer, Ludwig: Medizinische Spektrophotometrie, Jena, Gustav Fischer, 1933, p. 86.

tained 875 mg. of sodium chloride, 625 mg. of sodium citrate, 25 mg. of potassium chloride and 0.5 mg. of magnesium sulfate.[13]

The blood and the preservative were checked for initial p_H values, which were compared with the p_H of the mixture at the end of the experiment.

Hemoglobin determinations were done at the first suggestion of hemolysis. Cultures were taken at well spaced intervals.

The zero value used in determining the daily increments of hemoglobin was 40 mg. in the plasma from 100 cc. of blood. This quantity is our normal value for blood which has been centrifuged for one hour.

The basic values were as follows: hematocrit reading, 45.6 per cent cells and 54.4 per cent plasma; plasma potassium, 17.6 mg., whole blood potassium, 192.0 mg. and cell potassium (calculated), 400.0 mg. per hundred cubic centimeters; specific gravity of plasma, 1.0253; plasma proteins, 6.25 Gm. per hundred cubic centimeters; p_H of blood, 7.3 and p_H of preservative, 7.4

The results are tabulated in table 7.

Experiment 12.—*Blood preserved in sodium citrate and adrenal cortex extract.* The blood of the professional donor, J. F., was of group O. To 125 cc. was added 15 cc. of a 3 per cent solution of sodium citrate and 10 cc. of adrenal cortex extract, the mixture being treated like that of experiment 11.

Table 7 (experiment 11).—*Blood Preserved in Diluted Russian Citrate Compound*

Date	Days	Milligrams of Potassium: Observed Value per 100 Cc. of Plasma	Milligrams of Potassium: Sum of Increments: Per 100 Cc. of Blood	Milligrams of Potassium: Sum of Increments: From 100 Cc. of Cells	Percentage of Cell Potassium Diffused Out	Mg. of Hemoglobin in Plasma per 100 Cc. of Blood	Culture (Blood Agar)
9/23/38	1	19.2	9.7	21.2	5.3	..	0
9/24/38	2	26.3	20.5	44.9	11.2	..	..
9/25/38	3	27.8	22.8	50.0	12.5	..	..
9/26/38	4	37.3	37.0	81.0	20.3	..	..
9/27/38	5	40.1	41.4	90.0	22.5	..	0
9/28/38	6	47.7	52.3	114.7	28.7	..	..
9/30/38	8	50.3	56.1	123.0	30.7	..	..
10/ 2/38	10	53.6	60.9	133.5	33.4	..	..
10/ 4/38	12	60.9	71.4	156.5	39.1	..	..
10/ 6/38	14	67.5	80.7	177.0	44.3	..	..
10/ 8/38	16	74.1	90.0	197.4	49.3	..	0
10/10/38	18	80.1	98.4	215.8	54.0	..	..
10/12/38	20	81.9	100.8	221.0	55.3	0.7	..
10/14/38	22	84.0	103.8	227.5	56.9	193.0	..
10/16/38	24	92.7	115.7	253.7	63.4	341.0	..
10/18/38	26	100.3	126.0	276.0	69.0	533.0	0
10/20/38	28	106.5	134.3	294.6	73.6	692.0	..
10/22/38	30	107.3	135.4	296.8	74.2	813.0	..
10/24/38	32	109.0	137.7	302.0	75.5	1,172.0	..
10/31/38	39	111.0	140.2	307.5	76.9	1,708.0	..
11/ 7/38	46	123.0	155.5	342 0	85.4	2,242.0	..
11/14/38	53	128.3	162.3	356.0	89.0	3,157.0	0
11/18/38		$p_H = 7.31 \pm 0.05$ as determined by glass electrode					

Experiment 13.—*Blood preserved in undiluted Russian citrate compound.* The blood of the professional donor, J. F., was of group O. The procedure was similar to that of experiment 11 except that only one half the quantity of preservative was used and no distilled water was added. The 12.5 cc. of preservative contained 312.5 mg. of sodium citrate, to make a mixture containing 0.228 Gm. per hundred cubic centimeters of blood. The results are shown in table 9.

Experiment 14.—*Blood preserved in Grey's buffered solution.* The blood of the professional donor, J. F., was of group O. The procedure was the same as that in experiment 11 except that 112.5 cc. of blood was used and 12.5 cc. of preservative prepared according to Grey.[15] The results are shown in table 10.

Table 11 shows at a glance the difference in rates of hemolysis in the four types of preservative. The increases of each are expressed in grams in the plasma of 100 cc. of blood and then as percentage of hemoglobin lost from the cells.

Table 12 expresses the percentage loss of potassium from cells.

The information gained shows that none of the preservatives prevented an increase in plasma potassium,

Table 11.—*The Difference in Rates of Hemolysis*

(Samples collected Sept. 22)		Experiment 11; Diluted Russian Citrate Compound: Hemoglobin in Plasma		Experiment 12; Solution of Sodium Citrate and Adrenal Cortex Extract: Hemoglobin in Plasma		Experiment 13; Undiluted Russian Citrate Compound: Hemoglobin in Plasma		Experiment 14; Grey's Buffered Solution: Hemoglobin in Plasma	
Date	Days	Gm.	Per Cent	Gm.	Per Cent	Gm.	Per Cent	Gm.	Per Cent
10/12/38	20	0.001	0.005	0.078	0.32	0.115	0.76	0.025	0.17
10/14/38	22	0.175	1.17	0.094	0.63	0.164	1.09	0.078	0.52
10/16/38	24	0.344	2.29	0.190	1.27	0.259	1.73	0.116	0.77
10/18/38	26	0.524	3.49	0.254	1.69	0.354	2.36	0.164	1.09
10/20/38	28	0.684	4.56	0.376	2.51	0.477	3.18	0.238	1.59
10/22/38	30	0.812	5.41	0.502	3.35	0.631	4.34	0.342	2.28
10/24/38	32	1.150	7.67	0.716	4.77	0.981	6.54	0.436	2.91
10/26/38	34	1.319	8.79	0.802	5.35	1.133	7.55	0.598	3.99
10/28/38	36	1.488	9.92	0.888	5.92	1.285	8.57	0.760	5.07
10/30/38	38	1.697	11.31	1.223	8.15	1.493	9.95	1.061	7.07
11/ 7/38	46	2.236	14.91	1.674	11.60	2.165	14.03	1.463	9.75
11/14/38	53	3.135	20.90	2.151	14.34	3.526	23.50	1.673	11.15

and of the preservatives analyzed sodium citrate functioned best. The blood kept under oil manifested the same changes. The reason underlying the mechanism of potassium loss is obscure; the finding of an increase in ammonia may prove significant.

Amberson [16] has shown that hemoglobin itself is not toxic to the vertebrate body if it has been freed from stromas, and if the solution was properly balanced infusions containing from 12 to 14 per cent caused no abnormal reaction. The quantities present in the plasma of the most hemolyzed bloods under our observation have not approached this figure.

Table 12.—*Percentage Loss of Potassium from Cells; Comparison of Different Preservatives*

Days	2.5% Solution of Sodium Citrate	3% Solution of Sodium Citrate	Russian Citrate Compound	Peyton Rous Compound	Diluted Russian Citrate Compound	Solution of Sodium Citrate and Adrenal Cortex Extract	Undiluted Russian Citrate Compound	Grey's Buffered Solution
7	18.7	18.8	23.1	19.0	30.5	20.4	26.2	28.1
14	32.8	28.7	40.9	37.2	44.3	34.1	36.2	42.3
21	44.3	40.8	60.0	44.2	56.1	51.7	49.5	58.3
30	48.7	44.7	66.0	53.4	74.2	62.3	58.1	61.2

These potassium levels were confirmed in the laboratory of Dr. Robert F. Loeb on samples submitted Oct. 4, 1938.

SUMMARY, PART II

1. The erythrocytes of preserved blood lose potassium at different rates, depending in part on the type of preservative. This loss begins before the diffusion of hemoglobin. Hence the degree of hemolysis cannot be used as an index of potassium loss.

2. A high degree of potassium diffusion may be present in the complete absence of hemolysis (table 7).

III. THE TOXICITY OF POTASSIUM

With the observed increase of potassium in the plasma of stored blood, a reinvestigation of the toxic action of this base was deemed necessary.

15. Grey, Temple: Buffered Citrate Solution in Blood Transfusion, Lancet **2**: 1431 (Dec. 18) 1937.

16. Amberson, W. R.: Blood Substitutes, Biol. Rev., Cambridge Philosophical Soc. **12**: 48-86, 1937.

The literature on the subject is abundant,[17] and some of the lethal doses are tabulated in tables 13 and 14.

Six years after Blake's[2] discovery that injections of potassium caused cardiac arrest in dogs, Bouchardat and Stuart-Cooper[18] in a series of fifty experiments established the lethal doses for fish, frogs, fowl, dogs and rabbits. Although this work was done in 1846,

TABLE 13.—*Lethal Doses of Potassium (Gustav Bunge, 1871)*

Animal	Weight Kg.	Dose, Gm.	Salt	Time of Death	Authority
1. Introduced into Stomach					
Rabbits	...	3	KCl	30 min.	Guttmann
		1.6-4	KCl	40-70 min.	Bunge
Dogs	6	16-20	KCl	1 hr.	Podkopaew
		48	KNO_3	1½ hrs.	Orfila
2. Subcutaneous Injection					
Rabbits	...	1-1.5	KCO_3, KCl, KNO_3	15-20 min.	Guttmann
	1.2	4			
	2.0		KCl, KNO_3	47-355 min.	Falck
	1.2	3	KCl	1½ hrs.	Bunge
Cats	...	8	KCl	1¼ hrs.	Bunge
3. Intravenous Injection					
Rabbits	...	0.23	KCl	Immediate	Grandeau
Dogs	...	0.3	KNO_3	Immediate	Traube
		1-1.5	KCl	Immediate	Grandeau
		0.1	KCl	Immediate	Bunge
		0.6-1	KCl	.....	Podkopaew
4. Intra-Arterial Injection					
Dogs	...	1.5	KCl	.....	Podkopaew

the conclusions are valid today, namely that the toxic action of potassium depends on the mode of administration, the rate of injection, the amount of potassium in the salt used and the individual resistance of the animal.

Concerning the toxicologic effects of potassium there are many contradictory data, owing in part to the choice of the animal. There is, however, fairly uniform agree-

TABLE 14.—*Lethal Intravenous Doses of Potassium (Since Bunge)*

Year	Authority	Animal	Salt	Mg. of Potassium per Kg.
1881	Feltz and Ritter	Dog	KCl	48
1883	Bochefontaine	Dog	KCl	100
1892	Dogiel	Dog	KNO_3	20
1906	Bouchard and Oliver	Dog	KCl	39.90
1910	Joseph and Meltzer	Dog	KCl	88.3
1938	Marenzi	Dog	KCl	20

ment that small doses of potassium increase, while large doses weaken and paralyze, the normal functions of the nervous, glandular and muscular systems.

Most authorities attribute death to cardiac paralysis: Claude Bernard,[19] Traube,[20] Grandeau,[21] Aubert and Dehn,[22] Bohm,[23] Dogiel,[24] Feltz and Ritter,[25] Bochefontaine,[26] Binet,[27] Hald,[28] Mathison,[29] Howell,[30] Gross [31] and Wiggers.[32]

Electrocardiograms taken in the course of conditions associated with hyperpotassemia [33] or after ingestion,[34] injection,[35] perfusion [36] or topical application [32] of potassium salts show a variety of changes. These range from slowing of the rhythm, decrease in PR interval and low voltage, to bundle branch block, ventricular fibrillation and cardiac arrest.

The respiratory and cardiac centers are other focal points of potassium action. Hooker[37] had demonstrated, by infusing the medulla of dogs, that an increasing concentration of potassium over calcium in the spinal fluid is followed by both respiratory and cardiac arrest.

Vascular smooth muscle is constricted by an increase in potassium concentration.[38] Of importance is the recent experimental work of Katz and Linder,[39] who reported coronary dilatation with small doses and complete occlusion with larger doses of potassium. In this condition, sodium salts caused relaxation of the constricted vessels.

The effect of potassium on blood pressure depends on the manner of injection and amount of the salt injected.[40] In general a decline in blood pressure occurs with intravenous [41] and a rise with intra-arterial medication.[42]

Aubert and Dehn,[22] however, reported a rise with very small intravenous doses. Mathison[29] has confirmed both the pressor and depressor effects of potassium salts.

Toxic oral doses are promptly vomited, and with certain animals ligation of the esophagus [43] was necessary to demonstrate that absorption from the gastro-

17. Webster, R. W., and Brennan, W. A.: Potassium and Tartrates, Chicago, Commonwealth Press, 1927.

18. Bouchardat and Stuart-Cooper: Expériences sur l'action physiologique comparée des chlorure, bromure et iodure du potassium, Arch. gén. de méd. supp. 361-378, 1846.

19. Bernard, Claude: Leçons sur les effets des substances toxiques et médicamenteuses, Paris, J.-B. Baillière & Fils, 1857, p. 350.

20. Traube: Ueber die Wirkung des salpetersauren Kali auf das Herz, Centralbl. f. d. med. Wissensch. **2**: 429, 1864.

21. Grandeau, Louis: Expériences sur l'action physiologiques des sels de potassium, de sodium et de rubidium, J. de l'anat. et de physiol. **1**: 378-385, 1864.

22. Aubert, H., and Dehn, A.: Ueber die Wirkungen des Kaffees, des Fleischextracts und der Kalisalze auf Herzthätigkeit und Blutdruck, Arch. f. d. ges. Physiol. **9**: 115-155, 1874.

23. Bohm, R.: Ueber Wiederbelebung nach Vergiftungen und Asphyxie. Arch. f. exper. Path. u. Pharmakol. **2**: 68-101, 1878.

24. Dogiel, J.: Ueber den Einfluss einiger Kalisalze, auf Muskeln und Nerven, Centralbl. f. d. med. Wissensch. **30**: 354-356, 1892.

25. Feltz, V. T., and Ritter, C. E. E.: De l'urémie expérimentale, Rev. méd. de l'est. **13**: 176-177, 1881.

26. Bochefontaine, Louis Théodore: Quelques expériences relatives à l'étude des effets physiologiques de la soude, de la potasse et leurs sels, Compt. rend. Soc. de biol. **5**: 78-82, 1883.

27. Binet, P.: Sur la toxicité comparée des métaux alcalines et alcalino-terreux, Compt. rend. Acad. d. sc. **115**: 251-253, 1892.

28. Hald, P. T.: Die Wirkung der Kalisalze auf die Kreislaufsorgane, Arch. f. exper. Path. u. Pharmakol. **53**: 227-260, 1905.

29. Mathison, G. C.: The Effects of Potassium Salts upon the Circulation and Their Action on Plain Muscle, J. Physiol. **42**: 471-494, 1911.

30. Howell, W. H.: An Analysis of the Influence of the Sodium, Potassium and Calcium Salts of the Blood on the Automatic Contractions of Heart Muscle, Am. J. Physiol. **6**: 181-206, 1901-1902.

31. Gross, E.: Die Bedeutung der Salze der Ringer'schen Lösung für das isolierte Saugethierherz, Arch. f. d. ges. Physiol. **99**: 264-322, 1903.

32. Wiggers, C. J.: Monophasic and Deformed Ventricular Complexes Resulting from Surface Application of Potassium Salts, Am. Heart J. **5**: 346-350 (Feb.) 1930.

33. Scudder, J.; Zwemer, R. L., and Truszkowski, R.: Potassium in Acute Intestinal Obstruction, Surgery **1**: 74-91 (Jan.) 1937. Scudder and Zwemer.[37]

34. Scudder, J.; Zwemer, R. L., and Whipple, A. O.: Acute Intestinal Obstruction: Evaluation of Results in 2,150 Cases, with Detailed Studies of Twenty-Five Showing Potassium as a Toxic Factor, Ann. Surg. **107**: 161-197 (Feb.) 1938.

35. Chamberlain, F.; Scudder, J., and Zwemer, R. L.: To be published.

36. Gautrelet, Jean: De l'action sur le coeur de l'ion potassium dissocié et introduit par electrolyse, Compt. rend. Soc. de biol. **62**: 1084-1085, 1907.

37. Hooker, D. R.: The Perfusion of the Mammalian Medulla: The Effects of Calcium and of Potassium on the Respiratory Centers, Am. J. Physiol. **38**: 200-208, 1915.

38. Astolfoni, G.; Ricerche intorno all'azione farmacologica delle soluzioni dei sali de potassio, Arch. internat. de pharmacod. **11**: 313-356, 381-403, 1903. Hald.[28]

39. Katz, L. N., and Linder, E.: The Action of Excess Na, Ca and K on the Coronary Vessels, Am. J. Physiol. **124**: 155-160 (Oct.) 1938.

40. McGuigan, H. A., and Higgins, J. A.: Changes in the Circulatory Effect of Potassium Salts Due to Epinephrine, Am. J. Physiol. **114**: 207-211 (Dec.) 1935. Mathison.[29]

41. Kleeberg, F.: On the Influence of Potassium Sodium Tartrate upon the Circulation, Am. Med. **6**: 622-625, 1903. Aubert and Dehn.[22]

42. Mathison.[29] McGuigan and Higgins.[40]

43. Bunge, Gustav: Ueber die physiologische Wirkung der Fleischbrübe und der Kalisalze, Arch. f. d. ges. Physiol. **4**: 235-282, 1871. Orfila, M. P.: Traité des poisons, ed. 2, Paris, Crochard, 1818.

intestinal tract may be toxic. The lethal doses, however, are from seventy to a hundred times the intravenous ones.

ANIMAL EXPERIMENTS

To test again the toxic action of potassium, the rabbit and dog were selected. The former has a high and the latter a low potassium content of the blood cells.

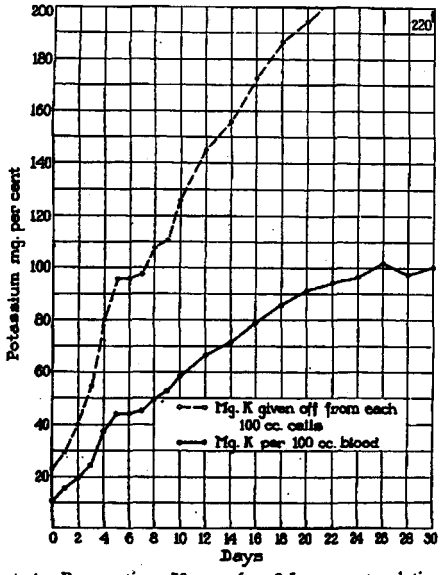

Chart 4.—Preservative: 70 cc. of a 2.5 per cent solution of sodium citrate for each 500 cc. of blood. The diffusion of potassium is expressed in two ways: (*a*) the amount given off from 100 cc. of cells (theoretical) and (*b*) the increase in the potassium in each hundred cubic centimeters of blood. Data from experiment 7, table 3. The points for this chart were obtained by adding the initial base line values to the daily increments expressed in the table. This chart shows the actual amount of potassium in each hundred cubic centimeters of blood. In giving a transfusion of 1,000 cc. of this blood on the thirtieth day one would be giving 1 Gm. of potassium. Under such circumstances extreme caution would need to be exercised, for example in the rate of injection.

TABLE 15.—*Rabbits Given Injections of Solution of Potassium Chloride*

Rabbit	Weight, Kg.	Concentration of Solution as Gm. per 100 Cc.	Amount Injected as Cc.	Place and Speed of Injection	Potassium Chloride as Gm.	Potassium as Mg. per Kg.	Results
1	2.01	0.381	550	Peritoneum Slowly	2.1	547	Died on seventh day
2	2.0	0.381	500	Peritoneum Slowly	1.96	514	Revived and given another injection on twelfth day
3	2.5	0.381	40	Jugular vein Rapidly	0.152	32	Convulsion; died at once
4	2.36	0.381	40	Jugular vein Rapidly	0.152	34	Convulsion; died at once
5	2.0	0.381	50	Jugular vein Rapidly	0.19	49	Convulsion; died in few minutes
2	2.0	1.16	42	Jugular vein Slowly	0.487	128	Convulsion; died in 15 min. 5 sec.

In our first set of experiments five rabbits were used. In three the potassium chloride solution was injected rapidly into the jugular vein, with immediate death after a generalized convulsion. In two the solution was injected into the peritoneum; one lived a week and the other recovered and was given an intravenous injection on the twelfth day. The results are given in table 15.

In the second group of experiments four dogs were used in an attempt to determine more accurately what part the speed of injection plays in the production of toxic symptoms. A typical protocol is presented in table 16. The actual lethal dose was given when 275 cc. (138.5 mg. of potassium per kilogram) of fluid had run in. The additional 25 cc. was accidental and may account for the excessive rise shown by the last value for plasma potassium. The gradual fall in the specific gravity and protein content of the plasma may be considered as an indication of blood dilution. The changes in the cell volume as recorded by hematocrit readings are more difficult to explain. The initial fall represents dilution and the secondary rise a sudden transport of fluid from the blood.

A summary of the experiments on dogs is presented in table 17.

TOXICITY IN MAN

The literature is very meager regarding the toxic action of potassium on man. Orfila [42] in 1818 reported the following cases:

A man with periodic fever took 1½ ounces (45 cc.) of potassium nitrate, thinking it was epsom salt, and died in ten hours.

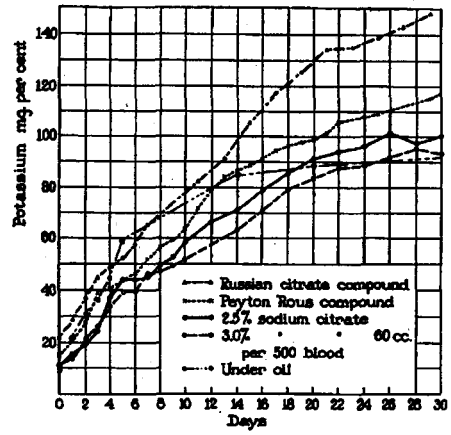

Chart 5.—Comparison of increase in plasma potassium in differently preserved bloods. The actual amounts of potassium in the plasma of 100 cc. of blood on any given day are shown. The points were obtained as in chart 4. The data are from experiments 1, 7, 8, 9 and 10, tables 1, 3, 4, 5 and 6.

A woman aged 40, suffering from heartburn, took 3 or 4 drachms (11 to 15 Gm.) of potassium sulfide in 4 ounces (120 cc.) of water by mistake. Severe vomiting ensued, followed by unconsciousness, the presence of black blood in the capillary system, especially of the lips and eyelids, and paralysis of the left side of the body. The action of the heart was barely perceptible and then failed. Autopsy showed the mouth and esophagus to be clean; the gastric mucosa was not greatly involved, except that here and there it was dry and red, with sulfur precipitates.

Many other cases were reported; when death was rapid the picture was one of shock and when it was delayed one of severe gastro-enteritis.

Bunge [43] observed that small doses do not affect the pulse or temperature.

Kylin [44] in 1925 injected from 0.15 to 0.8 Gm. of potassium chloride intravenously and reported a fall in the blood sugar content.

44. Kylin, E., and Engel, A.: Ueber die Einwirkung der K-ionen auf den Blutzuckerspiegel, Klin. Wchnschr. 4:653, 1925.

Arden [45] in 1934 recorded a case in which 15 Gm. of a potassium salt was taken by mouth. Muscular weakness, paresthesia of the hands and feet and a metallic taste in the mouth appeared in forty minutes and lasted from three to four hours.

Electrocardiographic tracings after the ingestion of potassium salts equivalent to 4.3 Gm. of potassium element have been reported.[34]

5. Recovery may be effected by artificial respiration, cardiac massage, the use of oxygen, injections of salt solution [46] and administration of cortical extract in large doses.[35]

COMMENT

The employment of preserved blood has increased during the past twenty years because of its numerous advantages.[47]

TABLE 16.—*Infusion into Dog of Isotonic (1.16 Gm. per Hundred Cubic Centimeters) Solution of Potassium Chloride*

Time	Elapsed Time in Minutes	Infusion	Hematocrit Reading; Percentage of Cells	Specific Gravity of Plasma	Plasma Proteins, Gm. per 100 Cc.	Whole Blood Potassium as Mg. per 100 Cc.	Plasma Potassium as Mg. per 100 Cc.	Source of Blood	Comment
10:25 a. m.	...	Before anesthesia	49.0	1.0262	6.56	24.5	22.7	Leg vein	Blood for base line slightly hemolyzed; given 30 mg. per Kg. of pentobarbital sodium
10:35 a. m.	0	After anesthesia; infusion started	44.6	1.0248	6.09	20.1	14.0	Leg vein	
10:44 a. m.	9	After 100 cc.	38.9	1.0240	5.81	24.2	20.2	Jugular vein	Rate of infusion 20 drops a minute after first 50 cc.
11:29 a. m.	54	After 200 cc.	40.2	1.0237	5.72	36.8	40.1	Jugular vein	Cyanotic, with irregular paired respirations, during a period when fluid went in too fast
11:39 a. m.	64	After 275 cc.	47.4	1.0281	5.52	49.2	62.1	Jugular vein	Convulsion, stopped breathing, sphincters relaxed; heart fibrillating
11:41 a. m.	66	After 300 cc.*	38.6	1.0195	4.28	67.0	172.0	Right side of heart	Heart stopped; blood clear; no hemolysis

At death: Potassium in cerebrospinal fluid from basal cistern, 51.8 mg. per hundred cubic centimeters and in urine, 74.4 mg. per hundred cubic centimeters

Autopsy heart dilated, no pericardial effusion, bladder full; no petechial hemorrhages

* 25 cc. accidentally run in rapidly after convulsion.

TABLE 17.—*Dogs Given Injections of Isotonic (1.16 Gm. per Hundred Cubic Centimeters) Solution of Potassium Chloride*

Dog	Weight, Kg.	Solution Injected as Cc.	Potassium Chloride Injected as Gm.	Potassium as Mg. per Kg. of Body Weight	Time, Minutes	Plasma Potassium Before Injection, Mg. per 100 Cc.	Plasma Potassium at Death, Mg. per 100 Cc.	Comment
1	9.8	100	1.16	62.1	3½	18.9	99.5	Pentobarbital sodium anesthesia, 30 mg. per Kg.; fluid run rapidly into vein by infusion; died after convulsion
2	10.3	120	1.392	73.0	18½	22.8	51.5	No anesthesia; fluid administered by syringe, 10 cc. at a time, slowly; died after a convulsion
3	8.8	100; some leakage into tissue	1.16	61.0	33	18.8	26.0	Pentobarbital sodium; infusion; young dog; died suddenly
						15.5	23.6	Spinal fluid
4	12.0	275	3.19	139.0	64	22.7	172.0	Pentobarbital sodium; fluid run in slowly by infusion; died after convulsion
							51.0	Spinal fluid

SUMMARY, PART III

1. The parenteral administration of potassium is associated with toxic manifestations of both muscular and nervous tissue together with a depression of the central nervous system.

2. The almost specific action of potassium is on the heart and circulation, with disturbances varying from diminished cardiac output to immediate diastolic arrest.

3. We have reaffirmed the lethal doses for animals previously reported.

4. The rate of injection is of particular importance, for when the potassium is given slowly several times the usual lethal dose is tolerated.

Of the many preservatives used, none tested prevented the diffusion of potassium from cells. In several hemolysis was slower and in one it did not take place at all, yet the diffusion of potassium was not altered. The remainder showed definite hemolysis; in some it started as early as the fifth day. All this illustrates the inadequacy of present methods of storage.

45. Arden, Felix: Experimental Observations upon Thirst and on Potassium Overdosage, Australian J. Exper. Biol. & M. Sc. **12**: 121-122 (Sept.) 1934.

46. Amberg, Samuel, and Helmholz, H. F.: The Detoxifying Action of Sodium Salt on Potassium Salt in the Guinea Pig, J. Pharmacol. & Exper. Therap. **12**: 19-35 (Aug.) 1918.

47. Lewisohn, Richard: Blood Transfusion by the Citrate Method, Surg., Gynec. & Obst. **21**: 37-47, 1915; A New and Greatly Simplified Method of Blood Transfusion, M. Rec. **87**: 141-142, 1915; Twenty Years' Experience with the Citrate Method of Blood Transfusion, Ann. Surg. **105**: 602-609 (April) 1937. Shamov, V. N.: The Transfusion of Stored Cadaver Blood, Lancet **2**: 306-309 (Aug. 7) 1937. Perry, Margaret C.: Preservation of Blood for Transfusion, Wisconsin M. J. **25**: 123-127 (March) 1926. Yudin, S. S.: Transfusion of Stored Cadaver Blood, Practical Considerations: The First Thousand Cases, Lancet **2**: 361-366 (Aug. 14) 1937. Robertson, O. H.: Transfusion with Preserved Red Blood Cells, Brit. M. J. **1**: 691-695 (June 22) 1918.

Although the observation that red blood cells lose potassium is not new,[48] no attempts have been made to prevent this loss. One of the problems of the future therefore is an attempt to keep blood under such conditions that both diffusion of the salts and hemolysis are prevented. The required knowledge may come through a better understanding of the laws which govern cell metabolism, diffusion and surface phenomena.

Naunyn [49] in 1868 first described the toxic properties of laked blood. His observations have been reaffirmed by many subsequent investigators, beginning with Landois,[50] but the nature of the toxic substance or substances has yet to be revealed.

Phemister [51] and Phemister and Handy [52] reported that there was something in slightly laked bloods which caused vasodilatation and in more severely traumatized blood which caused vasoconstriction. It was not histamine, not products of the disintegration of epinephrine and not the pituitary principle, nor did it develop as the result of changes in oxygen, carbon dioxide or hydrogen ion concentration, shifts in temperature or exposure to light or air. Petroff and his associates [53]

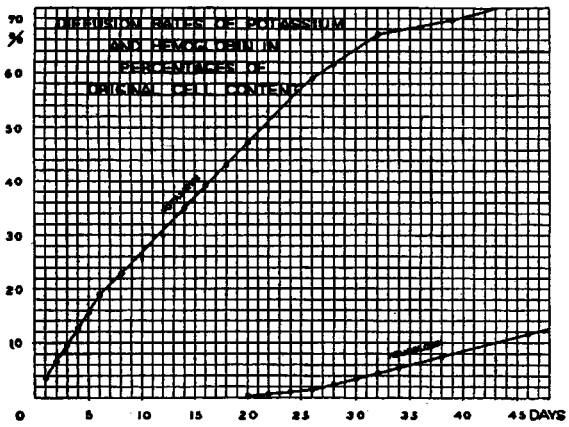

Chart 6.—Blood preserved in solution of sodium citrate and adrenal cortex extract. These curves illustrate that diffusion of potassium occurs before diffusion of hemoglobin and indicate the necessity of criteria other than hemolysis in assaying the various types of preservatives.

have observed such vasoconstriction of the splenic, renal and pulmonary vessels following the injection of hemolyzed blood but did not isolate the causative factors. Amberson [16] has established the fact that the toxic factor is not hemoglobin.

Kronecker [54] as long ago as 1882 suggested that the toxic effects of laked blood were due to its high potassium content. The finding of a large increase in the plasma potassium of preserved blood again raises this question. The intravenous lethal dose for man has not been observed. Studies on animals suggest that a rapid transfusion of from 3 to 5 liters of blood with a plasma content of 100 mg. per hundred cubic centimeters would be necessary to kill an adult. Blood preserved for thirty days by the methods now in use would contain potassium at this level, but it is unlikely that such quantities of stored blood would be used except as a slow continuous drip. It is probable, however, that such blood often may be used in quantities sufficient to cause toxic manifestations from its high potassium content.

The rapid administration of large quantities of blood preserved too long may be dangerous in conditions associated with hyperpotassemia, specifically in the dehydration of cholera,[55] intestinal obstruction,[56] intestinal fistula [57] and severe burns.[7]

Likewise its use seems contraindicated in both renal [58] and hepatic insufficiency [59] as well as in those diseases in which potassium retention is manifest, such as typhoid, influenza and pneumonia.[7]

In certain disorders of the ductless glands linked with disturbances in salt metabolism,[60] such as parathyroid tetany [61] and the collapse state of addisonian crises,[62] the giving of highly potassic blood may be dangerous.

On the other hand, in conditions of hypopotassemia, or potassium lack, preserved blood may find a specific use.

Finally, in hemorrhage and shock,[63] in which the need for blood is greatest and in which preserved blood finds its greatest usefulness, its improper use would seem to carry the greatest danger.

This study has been limited to potassium and hemoglobin. No claim is made that in potassium alone lies the noxiousness of preserved blood. Observations

48. Ashby, Winifred M.: A Study of the Mechanism of Change in Resistance of Erythrocytes to Hypotonic Salt Solution: III. A Study of the Cause of Effects Produced by Cations on the Resistance of Red Blood Corpuscles Previously Described, Am. J. Physiol. **63**: 585-610 (April) 1924. Kerr, S. E.: Studies on the Inorganic Composition of Blood: III. The Influence of Serum on the Permeability of Erythrocytes of Potassium and Sodium, J. Biol. Chem. **85**: 47-64 (Dec.) 1929. Ponder, Eric, and Robinson, E. J.: The Escape of Potassium from Rabbit Red Cells into Hypotonic Solution, Biochem. J. **28**: 1940-1943, 1934. Dulière.[1]

49. Naunyn, Bernard: Beitrage zur Lehre vom Icterus, Arch. f. Anat., Physiol. u. wissensch. Med. 1868, pp. 401-442.

50. Landois, Leonard: Die Transfusion des Blutes; Versuch einer physiologischen Begründung nach eigenen Experimental-Untersuchungen; Mit Berücksichtigung der Geschichte, der Indicationen, der operativen Technik und der Statistik, Leipzig, F. C. W. Vogel, 1875.

51. Phemister, D. B.: The Vascular Properties of Traumatized and Laked Bloods and of Blood from Traumatized Limbs, Ann. Surg. **87**: 806-810 (June) 1928.

52. Phemister, D. B., and Handy, J.: Vascular Properties of Traumatized and Laked Bloods, J. Physiol. **64**: 155-173 (Nov.) 1927.

53. Petroff, J.; Filatov, A.; Bogomolova, L., and Stroikova, X.: Experimentelle Untersuchungen über das Wesen des Hämolytischen Shocks bei der Bluttransfusion: Die Pathogenese der Veränderungen des Herzens und der Gefässe, Arch. f. klin. Chir. **181**: 209-226, 1934.

54. Kronecker, Hugo: Ueber die den Geweben des Körpers günstigen Flüssigkeiten, Deutsche med. Wchnschr. **8**: 261-263, 1882.

55. Schmidt, C.: Zur Characteristik der epidemischen Cholera gegenüber verwandten Transsudationsanomalieen, Leipzig, G. A. Reyher, 1850.

56. Cutler, E. C., and Pijoan, Michel: Certain Chemical Factors in Experimental High Intestinal Obstruction, Surg., Gynec. & Obst. **64**: 892-894 (May) 1937. Scudder, Zwemer and Truszkowski.[20] Scudder, Zwemer and Whipple.[24]

57. Scudder, J., and Zwemer, R. L.: The Effect of Complete Intestinal Fistula on Blood Potassium, Surgery **2**: 519-531 (Oct.) 1937.

58. Bouchard, Charles, and Oliver, Thomas: Lectures on Autointoxication in Disease or Self Poisoning of the Individual, ed. 2, Philadelphia, F. A. Davis Company, 1906. Olmer, Payan and Berthier.[4] Feltz and Ritter.[25]

59. Blum, L.; Aubel, E., and Hausknecht, R.: Les variations de la teneur du sang et des humeurs en sodium et en potassium après ingestion des sels de sodium et de potassium, Compt. rend. Soc. de biol. **85**: 498-500, 1921; Action diuretique des sels de calcium: Mécanisme de cette action, ibid. **85**: 950-952, 1921. Cantarow, Abraham: Changes in the Blood Following Repeated Withdrawal of Ascitic Fluid in Cirrhosis of the Liver, Am. J. Clin. Path. **8**: 142-152 (March) 1938. Dumitresco-Mante: La bradycardie et le syndrome humoral au cours des ictères du type catarrhal, J. de physiol. et de path. gén. **35**: 416-426 (June) 1937.

60. Baumann, E. J., and Kurland, S.: Changes in the Inorganic Constituents of Blood in Suprarenalectomized Cats and Rabbits, J. Biol. Chem. **71**: 281-302 (Jan.) 1927. Harrop, G. A.; Pfiffner, J. J.; Weinstein, A., and Swingle, W. W.: Further Studies on the Adrenal Cortical Hormone, Science **73**: 683-684 (June 19) 1931. Hastings, A. B., and Compere, E. L.: Effect of Bilateral Suprarenalectomy on Certain Constituents of the Blood of Dogs, Proc. Soc. Exper. Biol. & Med. **28**: 376-378 (Jan.) 1931. Loeb, R. F.: Chemical Changes in the Blood in Addison's Disease, Science **76**: 420-421 (Nov. 4) 1932. Marañón, G.; Collazo, J. A.; Barbudo, J., and Torres, Isabel: Metabolismo mineral y del agua en la enfermedad de Addison: ionemia e hidremia; hiperpotasemia en los accidentes graves, Arch. de med., cir. y especialid. **37**: 893-900 (Aug. 18) 1934. Marenzi, A. D.: Blood Potassium and Suprarenal Glands, Endocrinology **23**: 330-338 (Sept.) 1938. Zwemer, R. L., and Sullivan, R. C.: Blood Chemistry of Adrenal Insufficiency in Cats, Endocrinology **18**: 97-106 (Jan.-Feb.) 1934. Truszkowski and Zwemer.[9b]

61. Gross, E. G., and Underhill, F. P.: The Organic Ion Balance of the Blood in Parathyroid Tetany, J. Biol. Chem. **54**: 105-120 (Sept.) 1922.

62. Allers, W. D.; Nilson, H. W., and Kendall, E. C.: Studies on Adrenalectomized Dogs: The Toxic Action of Potassium, Proc. Staff Meet. Mayo Clin. **11**: 283-288 (April 29) 1936. Truszkowski, Richard, and Zwemer, R. L.: Experimental Alterations in Blood Potassium, Acta biol. exp. **12**: 1-12, 1938. Marañón, G., Collazo, Barbudo and Torres.[9b]

63. Schmitt, F., and Basse, W.: Einfluss des Aderlasses auf die Ionenverteilung zwischen Erythrocyten und Plasma bei Normalpersonen, Hypertonikern und Nephritiden, Arch. f. exper. Path. u. Pharmakol. **181**: 584-597, 1936. Thaler, J. I.: Evidence of Permeability of Tissue Cells to Potassium, Proc. Soc. Exper. Biol. & Med. **22**: 368-371 (Dec.) 1935. Zwemer, R. L., and Scudder, John: Potassium Changes in Experimental Shock, Am. J. Physiol. **119**: 427 (July) 1937; Blood Potassium During Experimental Shock, Surgery **4**: 510-527 (Oct.) 1938.

regarding the relative toxicities of magnesium,[64] ammonia and phosphorus will be reported subsequently.

CONCLUSIONS

1. There is a daily increase in the plasma potassium of preserved blood kept under aseptic, bacteriostatic conditions, with increments reaching as high as 1,000 per cent.

2. None of the preservatives tested prevent this diffusion of potassium from the cells, the lowest rates being found in blood preserved in a mixture containing 0.3 Gm. of sodium citrate per hundred cubic centimeters.

3. Agitation, such as shaking, hastens the loss of potassium from the cells.

4. The shape of the container markedly influences the rate of potassium diffusion; the greater the interface area between the cells and the supernatant plasma, the greater the rate of diffusion. This suggests that tubular containers may be preferable to wide-bottomed flasks.

5. There is no parallel between the rates of diffusion of potassium and hemoglobin; hence the degree of hemolysis cannot serve as an index of plasma potassium.

7. In pathologic states associated with potassium retention or sensitivity, the use of blood preserved too long seems ill advised.